Thematic Review Series: Glycosylphosphatidylinositol (GPI) Anchors: Biochemistry and Cell Biology

Trafficking of glycosylphosphatidylinositol anchored proteins from the endoplasmic reticulum to the cell surface

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Abstract In eukaryotes, many cell surface proteins are attached to the plasma membrane via a glycolipid glycosylphosphatidylinositol (GPI) anchor. GPI-anchored proteins (GPI-APs) receive the GPI anchor as a conserved posttranslational modification in the lumen of the endoplasmic reticulum (ER). After anchor attachment, the GPI anchor is structurally remodeled to function as a transport signal that actively triggers the delivery of GPI-APs from the ER to the plasma membrane, via the Golgi apparatus. The structure and composition of the GPI anchor confer a special mode of interaction with membranes of GPI-APs within the lumen of secretory organelles that lead them to be differentially trafficked from other secretory membrane proteins. In this review, we examine the mechanisms by which GPI-APs are selectively transported through the secretory pathway, with special focus on the recent progress made in their actively regulated export from the ER and the trans-Golgi network.—Muñiz, M., and H. Riezman. **Trafficking of glycosylphosphatidylinositol anchored proteins from the endoplasmic reticulum to the cell surface.** ***J. Lipid Res.*** **2016.** 57: **352–360.**

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The eukaryotic secretory pathway is responsible for the synthesis and delivery of correctly assembled proteins from the endoplasmic reticulum (ER) to their final functional destination, the extracellular media, the plasma membrane, or the endocytic/secretory membrane system. The vast majority of proteins are transported by a series of specific vesicular trafficking events (1). Many secretory proteins that are delivered to the cell surface, including a wide diversity of receptors, adhesion molecules, and enzymes, are attached to the external leaflet of the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor (2). The core structure of the GPI anchor precursor is largely conserved in evolution and consists of a phospholipid moiety (acylphosphatidylinositol) with a glycan backbone [Man4-(EtNP)Man3-(EtNP)Man2-(EtNP)Man1-GlcN], where EtNP is a side-branch ethanolamine-phosphate, Man is mannose (the numbers represent the positions of the Man in the anchor), and GlcN is glucosamine). Once the GPI anchor precursor has been made by a series of sequential reactions at the ER membrane, it is then attached en bloc in the ER lumen by a GPI-transamidase complex to newly synthesized proteins containing a GPI attachment signal sequence at their C terminus. Immediately after attachment to the protein, the structure of the lipid and glycan parts of the GPI anchor are modified by several remodeling enzymes (3). This remodeling process converts the GPI anchor into a transport signal that actively promotes the ER export of GPI-anchored proteins (GPI-APs) to the Golgi apparatus, from where they are subsequently routed to their functional site of residence, the plasma membrane.

The presence of the GPI anchor confers to GPI-APs a unique mode of membrane association within the lumen of secretory organelles that leads them to be transported

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Abbreviations: COPII (I), cytosolic coat protein complex II (I); DRM, detergent-resistant membrane; ER, endoplasmic reticulum; ERES, endoplasmic exit site; EtNP, ethanolamine-phosphate; GlcN, glucosamine; GPI, glycosylphosphatidylinositol; GPI-AP, glycosylphosphatidylinositol-anchored protein; Man, mannose; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; TGN, trans-Golgi network; v-SNARE, vesicle-associated SNARE.

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differently than transmembrane secretory proteins. Indeed, GPI-APs are segregated and sorted from other plasma membrane proteins along the secretory pathway (4–7). Thus, they constitute an exceptional system to study sorting mechanisms, and in particular, to understand the role of the interaction of secretory proteins with membrane lipids in their transport and sorting along the secretory pathway. In this sense, the study of the GPI-AP biosynthetic pathway has led to several novel concepts in membrane trafficking. In this review, we will examine the proposed mechanisms by which GPI-APs are selectively transported and sorted along the secretory pathway in different cell systems, focusing on the events that occur during the export from the ER and from the trans-Golgi network (TGN).

EXPORT FROM THE ER

To initiate the secretory pathway, correctly folded and assembled secretory proteins are selectively incorporated into protein-coated membrane vesicles that transport them from the ER to the Golgi apparatus. These vesicles are generated by polymerization of the cytosolic coat protein complex II (COPII), which locally bends the ER membrane at specific domains called ER exit sites (ERESs). For efficient ER export, most secretory proteins are actively captured by direct or indirect interactions with the COPII coat to be first concentrated at ERESs and then packaged into nascent COPII vesicles (8). At first, it was believed that all secretory proteins travel together in the same COPII vesicles to the Golgi, from where they are sorted to their final destinations. However, first chemical and genetic evidence, and then biochemical and morphological evidence, showed that in yeast, GPI-APs are segregated from other secretory proteins, in particular other plasma membrane proteins, already in ERESs and subsequently incorporated into distinct COPII vesicles (5, 7). The initial chemical and genetic experiments showed that GPI-APs had specific requirements for transport from the ER to the Golgi in yeast. Myriocin, an inhibitor of the first enzyme in ceramide biosynthesis, serine palmitoyltransferase, showed a rapid effect on the transport of only GPI-APs, suggesting that ongoing sphingolipid synthesis is required for their transport, but not for other secretory proteins. The *lcb1-100* temperature-sensitive mutant affecting this same enzyme, confirmed this finding, and additional mutants, including a mutant in the COPI coat, *ret1-1*, and a mutant in a conserved family of small proteins of the early secretory pathway, *emp24Δ* (9–11), also confirmed specific requirements for GPI-AP transport. Further exploration of the role of sphingolipid biosynthesis in GPI-AP transport in yeast showed that only stereoisomers of sphinganine that can be converted to ceramide were able to complement the *lcb1-100* mutation for GPI-AP transport, showing that ceramide synthesis was also necessary. This study also showed that ceramide synthesis was required for the stable membrane association of the GPI-APs (12).

Biochemical evidence in yeast demonstrated that GPI-APs leave the ER in distinct vesicles from other secretory proteins (5). ^{35}S-methionine and cysteine were used to label newly synthesized proteins in cells, which were then permeabilized and used for a cytosol-dependent in vitro budding assay. After vesicle formation, two independent isolation methods to separate the ER-derived vesicles were used; vesicle immuno-isolation and gradient separation. Both of these methods showed that plasma membrane amino acid transporters and GPI-APs were found in distinct ER-derived vesicles. On the other hand, pro-α factor, a soluble secretory protein, was co-isolated with the amino acid transporters. To complement the genetic and biochemical studies, a morphological approach provided definitive evidence for GPI-AP sorting at the level of ERESs (7). Using fluorescent protein-labeled GPI-APs and plasma membrane transporters together with a thermosensitive COPII mutant allele that accumulates secretory cargo in the ERESs at restrictive temperature, it was observed that GPI-APs accumulate in ERESs that are distinct from those in which other secretory proteins accumulate. Different GPI-APs could be colocalized to the same ERES, as could polytopic plasma membrane proteins, but the overlap of the two classes of proteins was minimal. The selective concentration of GPI-APs at specific ERESs and subsequent incorporation into distinct transport vesicles indicate the existence of at least two parallel pathways for routing secretory proteins from the ER to the Golgi in yeast.

In contrast to the remarkable segregation observed in yeast, in mammalian cells GPI-APs are not sorted from other secretory proteins upon COPII vesicle formation. Indeed, it has been shown that GPI-APs and transmembrane secretory proteins are found in the same ERESs and COPII vesicles (13). Recent findings strongly suggest that GPI-APs use different mechanisms to concentrate into COPII vesicles during exit from the ER in the two organisms. More importantly, the difference between these export mechanisms might explain the propensity of yeast GPI-APs to segregate from other secretory proteins upon the ER exit.

Lipid-based sorting of GPI-APs in yeast

In contrast to other secretory proteins, it seems that yeast GPI-APs do not require the COPII machinery for their concentration at ERESs (7). First, it is impossible for GPI-APs to interact directly with the COPII coat because GPI-APs are not exposed to the cytoplasm. Second, among the known adaptors that link luminal cargoes with the COPII coat, only mutants in the members of the p24 family, *emp24* and *erv25*, affect GPI-AP transport (10, 14). It has been clearly shown that these mutants do not affect GPI-AP concentration into ERESs (15). Therefore, an alternative mechanism for cargo concentration needs to function in this case. It seems that GPI-APs are most likely concentrated and sorted by a lipid-based mechanism, which involves the structural remodeling of the lipid moiety of the GPI anchor (7). This is consistent with the chemical and genetic evidence presented earlier. The process of GPI-anchor remodeling begins immediately after protein attachment to the GPI anchor and, in yeast, consists of inositol deacylation by Bst1p (16), followed by fatty acid remodeling, which involves the removal of the long-chain

TABLE 1. Intracellular localization of the GPI anchor remodeling enzymes in yeast and mammals

	Yeast		Mammals	
	ER	Golgi	ER	Golgi
GPI anchor remodeling enzymes				
GPI ethanolamine phosphate phosphoesterase	Ted1p	−	PGAP5	−
GPI inositol deacylase	Bst1p	−	PGAP1	−
GPI phospholipase A2	Per1p	−	−	PGAP3
GPI O-acyltransferase	Gup1p	−	−	PGAP2
Ceramide remodelase	Cwh43p	−	−	−
Resistant to detergent extraction	+	+	−	+
Ceramide synthesis and transport	C26 Cer	Mainly vesicular	C16-24 Cer	Vast majority nonvesicular
GPI-AP sorting event	+	?	−	+

Enzymes, properties, and events occuring in the secretory pathway. + indicates presence, − indicates absence.

unsaturated fatty acid at the sn2 position by Per1p (17) and its replacement with a very long-chain saturated fatty acid (C26) by Gup1p (18) (**Table 1**). In most cases, the C26 diacylglycerol formed as part of the anchor is replaced with a ceramide that also contains a very long-chain saturated fatty acid (C26), by Cwh43p (19, 20). In yeast, the entire remodeling process occurs in the ER and is a prerequisite for GPI-APs to efficiently exit from the ER, with the function of Cwh43p being somewhat less critical. Indeed, unremodeled GPI-APs generated in *bst1*, *per1*, or *gup1* mutants fail to be concentrated into their specific ERESs (7). In addition, GPI-anchor lipid remodeling is required for the isolation of GPI-APs as detergent-resistant membranes (DRMs) (15, 17, 21), which has been proposed to reflect their association to ceramide-enriched domains, although this interpretation of the experiments is debatable. Nevertheless, these results clearly show that GPI-anchor lipid remodeling changes the physical properties of the GPI-APs and perhaps their functional association with the membrane. The chemical and genetic experiments described above are consistent with the role of ceramide in GPI-AP transport. Because the GPI-AP transport phenotype associated with the *cwh43* mutation is much less severe than myriocin treatment or the *lcb1-100* mutation, it is highly likely that ceramide plays an additional role in GPI-AP transport besides its use as a substrate in anchor remodeling. In artificial membranes, biophysical experiments suggest that ceramides can coalesce to form platforms with specific physical properties (22). It is likely that the remodeled GPI-AP lipid moieties, with a very long saturated acyl chain at the sn2 position, have similar properties to ceramide. The current best hypothesis, in our opinion, is that ceramides and remodeled GPI-APs take advantage of their physical properties to coalesce into

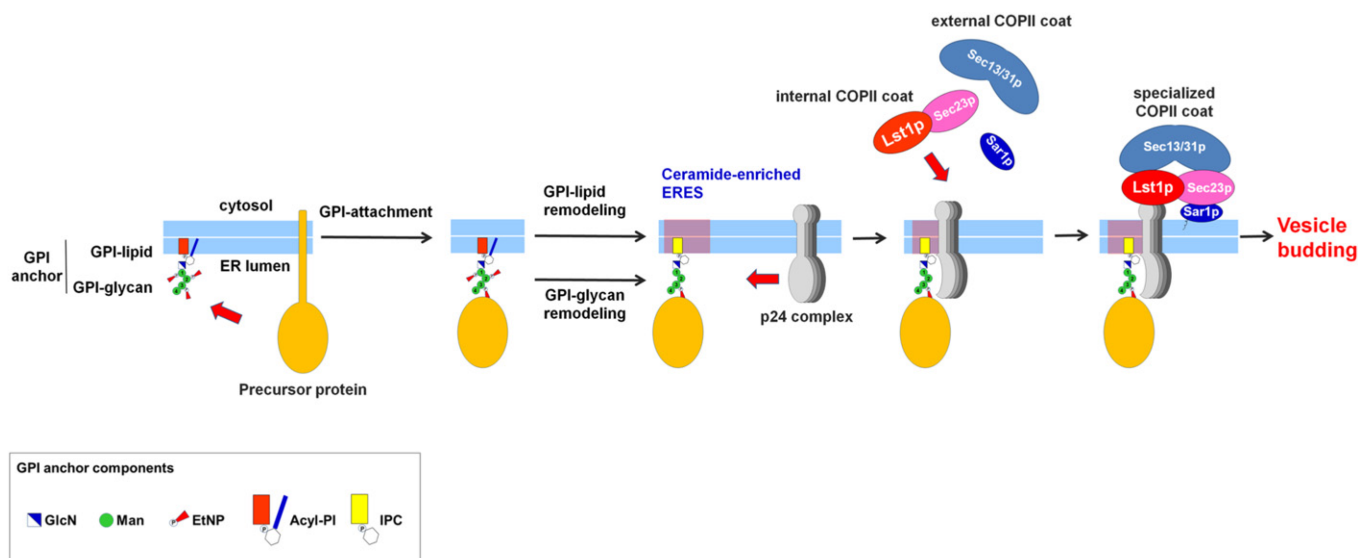


Fig. 1. In yeast, GPI-APs use a specialized COPII vesicle budding system for ER export, which is actively regulated by the structural remodeling of the GPI anchor. After protein attachment, lipid and glycan parts of the GPI anchor are remodeled. The GPI-lipid remodeling leads to the sorting and concentration of GPI-APs at specific ERESs. The GPI-glycan remodeling allows the subsequent recruitment of the p24 complex, which functions as a specific lectin by recognizing the remodeled GPI-glycan moiety of GPI-APs, to these ERESs. This lectin-based GPI-cargo binding stimulates the p24 complex to selectively recruit and stabilize Lst1p-Sec23p prebudding complexes, which afterwards favor the assembly of the external COPII layer to generate specialized COPII vesicles enriched in GPI-APs. Because GPI anchor remodeling occurs after protein attachment, this implies that COPII vesicle production is fine-tuned by the number of cargo proteins that are correctly anchored and ready for ER export. Acyl-PI, acyl-phosphatidylinositol; IPC, inositolphosphoceramide.

ordered domains in the relatively disordered lipid environment of the ER. These domains would be selectively concentrated at specific ERESs from where they can be cotransported to the Golgi in the same specialized COPII vesicles (**Fig. 1**). This is consistent with a previous hypothesis suggesting cotransport of ceramides and GPI-APs (23, 24). Whether there is a link between these ordered domains with an ERES with specific properties is still a mystery. However, an alternative and plausible explanation for localization to specific ERESs could be that the formation of highly ordered domains of ceramides and GPI-APs could provide a hostile membrane environment for the incorporation of transmembrane proteins, thus creating specific ERESs by exclusion. Upon formation of specific COPII vesicles enriched in GPI-APs, vesicle-associated soluble N-ethylmaleimide-sensitive factor attachment protein receptors (v-SNAREs) must be incorporated as they are required for subsequent vesicle fusion events. The exclusion mechanism is less likely to have an effect on SNARE proteins than on multi-spanning plasma membrane proteins, because they either span the membrane only once or, for some SNAREs, even not at all. Interestingly, some v-SNAREs and specific tethering factors have been shown to be required for sorting of GPI-APs upon the ER exit, although the underlying mechanism still remains elusive (25, 26).

Packaging of GPI-APs into ER-derived vesicles in yeast

Once GPI-APs have been selectively sorted and concentrated into specific ERESs upon GPI-anchor lipid remodeling, they must then be incorporated into COPII vesicles for transport to the Golgi. Due to their complete luminal topology, GPI-APs need a transmembrane cargo-coat adaptor or cargo-receptor to be indirectly connected with the cytosolic COPII coat if they are to be actively packaged into nascent COPII vesicles. This adaptor role for GPI-APs is achieved by the p24 proteins (15, 27, 28). The conserved p24 family members are abundant type I transmembrane proteins with a large luminal domain and a short cytoplasmic tail harboring COPII and COPI coat binding signals (29–32). The founding member of this family, Emp24p, was shown to be required for the efficient maturation of GPI-APs, but its mutation had less effect on several, but not all, other secretory proteins (10). Emp24p, as well as a second member of the family, Erv25p, have been identified as proteins enriched in COPII vesicles formed in vitro (14). They have been shown to function together. Indeed, the p24 proteins assemble in heteromeric complexes that cycle between ER and Golgi compartments (33). A large set of experimental evidence has shown that the yeast p24 complex is required for the selective ER-to-Golgi transport of GPI-APs and fulfills specific cargo receptor requirements: engaging newly synthesized GPI-APs with the COPII coat to drive their incorporation into the nascent COPII vesicles, traveling together to the Golgi (15, 27, 28).

Complementing the genetic experiments above that suggested a role for Emp24p in GPI-AP transport, both biochemical and morphological studies have been performed that confirm the link between GPI-APs and Emp24p. In vitro budding experiments showed that the *emp24* mutation reduced formation of vesicles containing GPI-APs without adversely affecting the formation of vesicles with other cargoes (28). Pretreatment of the membranes with antibodies against the cytoplasmic tail of Emp24p before the budding reaction had a similar effect. Morphological experiments showed that even though Emp24p was not required for concentration of GPI-APs at ERESs, mutants that were unable to concentrate GPI-APs showed a mislocalization of Emp24p to the ER and virtual absence from ERESs (15). These results strongly suggest that there is a functional association between Emp24p and GPI-APs, which is supported directly by cross-linking experiments (28).

If the Emp24p complex directly interacts with GPI-APs, what is recognized by the complex and how is this binding regulated? Recognition of GPI-APs by the p24 complex (containing Emp24p) in yeast has been recently shown to be directly regulated by the remodeling of the glycan portion of the GPI anchor (27). This remodeling process involves the removal of the initial side-chain EtNP on the second Man of the GPI-glycan by the specific phosphodiesterase, Ted1p (27, 34, 35) (Table 1). The GPI-glycan remodelase activity of Ted1p is required for GPI-AP recognition by the p24 complex in vivo. Furthermore, the p24 complex is able to bind, in vitro, a synthetic glycan that mimics the remodeled GPI-glycan, and this interaction can be out-competed by Man, but not glucose. Therefore, the p24 complex acts as a lectin by specifically recognizing the remodeled structure of the GPI-glycan moiety only after the EtNP has been removed by Ted1p (27). The same study also addressed how this lectin-based recognition is then coupled to the COPII coat assembly for ER export of GPI-APs. Interestingly, binding of the remodeled GPI-APs was found to induce the p24 complex to specifically recruit the COPII subunit, Lst1p, to ERESs (27). Lst1p is one of two paralogs of the major COPII cargo binding subunit, Sec24p (36, 37). Although the p24 proteins can interact with both Lst1p and Sec24p (37), GPI-APs are only connected by the p24 complex to Lst1p, but not to Sec24p (27). The binding preference of the p24 complex for Lst1p when it is bound to GPI-APs could be explained in two ways. First, cargo binding could trigger a structural change of the p24 proteins that selectively enhances their affinity for Lst1p, but not for Sec24p. Alternatively, a special lipid environment, such as the ceramide-enriched domains proposed to be associated with lipid remodeled GPI-APs, could play a role. It is possible that Sec23p/Lst1p subcomplexes of the COPII coat might prefer to bind to the ceramide-enriched domains postulated above. Interaction with p24 complexes bound to GPI-APs would then stabilize their recruitment to the specific ERESs (Fig. 1). In both cases, the p24 complex should show a certain degree of local activation, which might be supported by the fact that Lst1p and Sec24p were observed to be unevenly distributed at ERESs (38).

In order to ensure efficient vesicle formation and packaging of GPI-APs from their specific ERESs, Lst1p must be selectively recruited by the p24 complex (15, 39, 40). Indeed, recent data have shown that the local concentration

of both GPI-APs and p24 proteins at specific ERESs imposes special COPII coat scaffolding requirements for vesicle budding, such as the presence of Lst1p and more dependence on Sec13p, a subunit of the outer layer of the COPII coat (41, 42). Sec13p could confer sufficient rigidity to the coat in order to overcome the membrane-bending force potentially associated with GPI-AP-enriched membranes, because the size of the heavily glycosylated GPI-AP luminal domains are large in relation to their membrane anchors. In turn, Lst1p has been proposed to adjust this coat rigidity by creating a larger diameter vesicle bud, which is consistent with the observation that mixed Sec24p-Lst1p COPII vesicles formed in vitro are slightly larger than those vesicles formed only by Sec24p (43). Thus, Lst1p, together with Sec13p, functions to provide specialized structural scaffolding to the COPII coat, which would be required to encapsulate potentially larger cargos, such as clusters of GPI-APs and p24 proteins embedded into more rigid ceramide-enriched membranes (42). Interestingly, supporting this idea, a genetic screen looking for mutants that bypass the requirement for *SEC13* found mutants that are defective in GPI-AP remodeling and transport, including mutants in *BST1* and *BST2/EMP24* genes (44).

A remarkable aspect of this specialized COPII vesicle budding is that it is actively regulated by GPI anchor remodeling (27). Whereas GPI-anchor lipid remodeling sorts and concentrates GPI-APs at their specific ERESs (7), GPI-glycan remodeling subsequently promotes the recruitment of the specialized ER export machinery that enables vesicle formation from these ERESs (27). The p24 complex must first be recruited to specific ERESs, where it binds to concentrated remodeled GPI-APs. Next, this binding stimulates the p24 complex to selectively recruit and stabilize Lst1p to generate specialized COPII vesicles enriched in GPI-APs. Therefore, this specialized COPII system reacts to the presence of mature cargo, which functionally links luminal cargo maturation to COPII vesicle budding. Because GPI anchor remodeling occurs after protein attachment, this implies that COPII vesicle production is fine-tuned by the number of cargo proteins that are correctly anchored and ready for ER export.

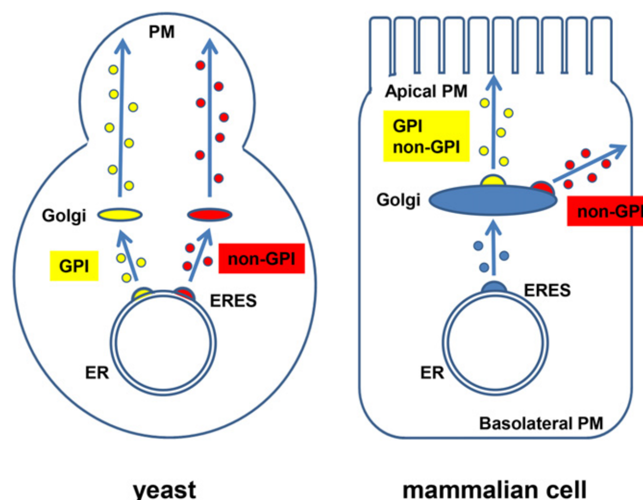


Fig. 2. GPI-APs are segregated and sorted from other types of cell surface proteins during their transport through the secretory pathway. This figure illustrates the known sites for sorting of GPI-APs in yeast and mammalian cell systems. In yeast, this sorting occurs upon the ER export. Newly synthesized GPI-APs are sorted and concentrated at specific ERESs from where they are subsequently incorporated into distinct COPII vesicles for Golgi delivery. The speculative possibility that GPI-APs continue to travel separately from other secretory proteins through different Golgi cisternae to the plasma membrane is shown, although this issue has not been experimentally addressed yet. In mammalian polarized epithelial cells, GPI-AP sorting takes place later, in the Golgi, during export from the TGN toward the apical plasma membrane (PM). GPI-APs and other apically-targeted proteins are segregated from basolateral proteins into different secretory vesicles that follow separate routes to the cell surface.

Mechanisms of GPI-AP export from the ER in mammalian cells

In mammalian cells, in contrast to yeast, GPI-APs are not segregated and sorted from other secretory proteins upon the ER exit, being incorporated in the same ERESs and COPII vesicles for delivery to the Golgi (13). This apparent discrepancy in mechanism can be explained by the fact that GPI-anchor glycan remodeling occurs in the ER, as in yeast, but lipid remodeling occurs in the Golgi (21, 45) (**Fig. 2**). This also correlates with differences in the ceramide population and mechanism of transport. Most mammalian cells have shorter (C16–C24) ceramides than yeast (46), and most of the mammalian ceramide transport is carried out by CERT, a cytosolic protein that picks up ceramide from the ER and delivers it directly to the Golgi (47). In yeast, most of the ceramide transport is via vesicular traffic (48). Therefore, GPI-APs in mammalian cells cannot use the lipid-based sorting mechanism to concentrate at ERESs. Consistent with this, unlike yeast, ER-to-Golgi transport of GPI-APs in mammalian cells does not depend on de novo sphingolipid biosynthesis (13). Nevertheless, it has been recently suggested that the transport of the longer ceramides (C24), but not the shorter ones, depends on GPI (24). In addition, it has been shown that cholesterol is required for the efficient ER export of GPI-APs, although their potential association with cholesterol is most likely not sufficient to sort GPI-APs into specific ERESs and COPII vesicles (49). In contrast to yeast, GPI-AP concentration at ERESs depends on the p24 complex (50). This is consistent with a COPII-mediated concentrative mechanism in mammalian cells, by which the cytosolic COPII coat captures luminal GPI-APs through the transmembrane p24 proteins to concentrate them in ERESs prior to budding.

Despite these differences between the mammalian and yeast p24 complex imposed by the GPI-anchor lipid composition, the mammalian p24 complex likely recognizes the GPI-AP in the same way as the yeast p24 complex. Ted1p is the ortholog of PGAP5 which has GPI-glycan remodelase activity, and PGAP5 is also required for GPI-AP recognition by the p24 complex (34, 50). Recent data has involved the membrane-adjacent α-helical region as the binding domain of the p24 proteins (51). Furthermore, both mammalian p24 and GPI-APs use the same specific isoforms of the COPII

cargo binding subunit, Sec24p (Sec24C and Sec24D), to exit the ER (49). Taken together, these findings suggest that the mammalian p24 complex could also link GPI-APs with the COPII coat for their efficient packaging in COPII vesicles, as has been shown in yeast.

Golgi arrival and post-ER quality control

During or after arrival to the Golgi, remodeled GPI-APs dissociate from the p24 complex, as seen from the fact that only the ER form of GPI-APs can be found associated with the p24 proteins (15). This dissociation could be caused by a decreasing pH in a later Golgi subcompartment that would induce conformational changes in the p24 proteins to lower their affinity for bound ligand. Indeed, it has been reported that the interaction of mammalian p24 proteins with GPI-APs depends on pH, which is consistent with a binding in the ER and dissociation in a post-ER acidic compartment, such as ERGIC or *cis*-Golgi (50). Upon their release, remodeled GPI-APs can progress through the secretory pathway to be finally delivered to the plasma membrane. Released p24 proteins can be recycled to the ER in COPI vesicles to initiate another round of ER export. The p24 proteins have been proposed to play an additional role in COP I budding from the Golgi (52–54). It has been reported in yeast that the retrieval of the p24 complex is involved in a post-ER quality control system that monitors the completion of anchor remodeling and contributes to the retention of unremodeled GPI-APs in the ER (15). The yeast p24 complex functions in the retrieval of escaped unremodeled GPI-APs from the Golgi to the ER in COPI vesicles. Although the precise mechanism of retrieval is still unclear, it has been shown that p24 interacts in a different manner with escaped unremodeled GPI-APs in the Golgi than with remodeled GPI-APs destined for ER export. Indeed, the p24 interaction with unremodeled GPI-APs can only be detected by using cross-linking reagents, but not by native co-immunoprecipitation in the presence of detergent. The lack of co-immunoprecipitation could be explained by a lower affinity and/or by a detergent sensitivity of p24 binding to the GPI-AP. It is possible that detergent could interfere with a p24 protein-GPI-anchor lipid interaction. Consistent with this idea, the p24 proteins from mammalian cells have been shown to directly bind specific sphingomyelins (54). Finally, once retrieved to the ER, unremodeled GPI-APs would have another opportunity to acquire a properly remodeled anchor. Thus, the yeast p24 complex senses the status of the GPI anchor, regulates GPI-AP intracellular transport, and coordinates this with correct anchor remodeling.

EXPORT FROM THE TGN

After being fully glycosylated during their passage through the Golgi cisternae, GPI-APs exit the Golgi from the TGN in secretory vesicles that transport them to the plasma membrane. In mammalian cells, GPI-APs are sorted from other secretory proteins at the level of the TGN, where they are segregated into different secretory vesicles that follow separate routes to the cell surface. This sorting step has been shown to occur in nonpolarized cells, although it has been better studied in polarized cells, such as neurons or epithelial cells, in which GPI-APs are predominantly sorted to the axon or to the apical domain of the plasma membrane, respectively (55–58). Nevertheless, there are exceptions to this rule, because in different epithelial cell lines some GPI-APs are sorted and transported basolaterally (59, 60). An especially interesting case is the GPI-anchored high density lipoprotein-binding protein 1 (GPIHBP1), which is a GPI-AP that transports lipoprotein lipase from the subendothelial spaces to the luminal face of capillary endothelial cells, being thus enriched in both the basolateral and apical plasma membrane domains of endothelial cells (61). Finally, in yeast, because GPI-APs are already sorted from other secretory proteins at the level of the ER, it is still unknown whether they continue traveling separately through distinct Golgi stacks to the plasma membrane or whether they mix in the same Golgi stacks to be sorted again in the TGN.

Mechanisms of sorting and export from the TGN

In most polarized epithelial cells, the GPI anchor appears to act as an apical sorting signal, because in these cells GPI-APs are delivered in specific secretory vesicles from the TGN to the apical, but not to the basolateral, membrane (62). Interestingly, this sorting step correlates with the acquisition of two saturated fatty acids by the GPI anchor through GPI-lipid remodeling in the Golgi and leads to the recovery of GPI-APs with DRMs (21, 45). Furthermore, in addition to the GPI-APs and other proteins, the apical membrane is also enriched in saturated lipids such as sphingolipids, which are made in the Golgi, and cholesterol (63, 64). These facts have led to the proposal of a lipid-based mechanism for apical sorting of GPI-APs from the TGN. It is postulated that GPI-lipid remodeling in the Golgi leads GPI-APs to cluster and associate through the two saturated fatty acids with sphingolipids and cholesterol into specialized lipid-ordered domains which serve as selective platforms for vesicle budding at the TGN (65, 66). Supporting this model, inhibitors of sphingolipid biosynthesis and/or removal of cholesterol impairs the apical sorting of GPI-APs (67, 68). However, the proposed role of GPI-lipid remodeling in apical sorting is not clear yet. Indeed, recent evidence indicates that unremodeled GPI-APs with unsaturated fatty acids are transported with the same efficiency to the plasma membrane as remodeled GPI-APs with saturated fatty acids (69). Furthermore, a previous study had shown that lyso forms of GPI-APs (GPI-lipid remodeling intermediates containing only one saturated fatty acid) are apically sorted independent of DRM association, although sorting is still sensitive to cholesterol depletion in polarized epithelial cells (70). These data suggest the possible existence of alternative pathways that redundantly regulate apical targeting. Nevertheless, further studies testing the behavior of native unremodeled GPI-APs (containing phosphatidylinositol) in polarized epithelial cells will be required to conclusively clarify the putative role of GPI-lipid remodeling in apical sorting of GPI-APs.

Additionally, another prediction of the lipid-based sorting mechanism from the TGN is that GPI-APs and saturated lipids should be co-sorted in secretory vesicles generated from the TGN. Consistent with this, it has been observed, using yeast fractionation, that purified Golgi-derived vesicles are enriched in sphingolipids and ergosterol (71). Nevertheless, this study cannot rule out the possibility that the observed lipid enrichment is the direct result of an earlier sorting that had already occurred upon ER exit.

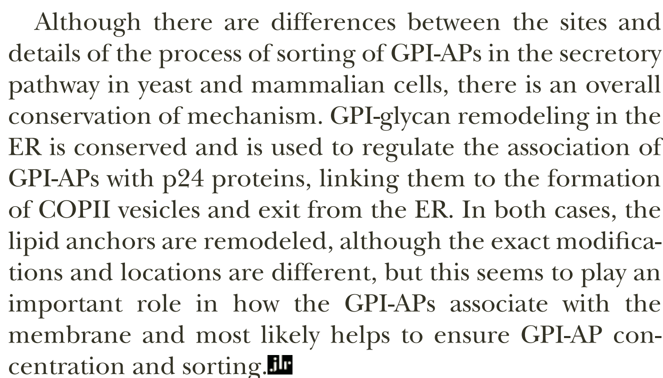
Alternatively, other mechanisms have been reported to contribute to the apical sorting of GPI-APs upon TGN export in different polarized epithelial cells. GPI-APs have the intrinsic property to form high molecular weight complexes during their transport to the apical membrane (68). This oligomerization process is required for apical sorting of GPI-APs, because its impairment results in the missorting of GPI-APs to the basolateral domain (68, 72). Interestingly, some specific GPI-APs are still delivered to the basolateral membrane, and those proteins do not oligomerize (73). The formation of apical GPI-AP oligomers takes place at the Golgi, as observed by pulse-chase experiments, at about the same time as GPI-lipid remodeling and GPI-AP association to DRM (68). Indeed, it has been shown that oligomerization depends on cholesterol in epithelial polarized cells and also requires fatty acid remodeling in nonpolarized cells, like fibroblasts (68, 74). These data suggest a role in GPI-AP oligomerization for GPI-GPI interactions in a saturated lipid environment. Furthermore, oligomers also rely on protein-protein interaction through the ectodomains of GPI-APs (68, 73). Oligomerization has been proposed to facilitate GPI-AP segregation from other secretory proteins and to promote the coalescence of small lipid domains into larger and more stable domains that would favor vesicle budding from the TGN (68). In polarized epithelial cells, the oligomerization-based sorting mechanism of GPI-APs in the Golgi acquires a tremendous physiological relevance because it regulates both their organization and function at the apical membrane (75).

N-glycosylation has been shown to be additionally required for apical delivery of GPI-APs, as well as other apically-targeted glycoproteins, suggesting an involvement of a lectin receptor-based mechanism (76). Galectins have been proposed to fulfill this role as apical sorting receptors at the TGN (77–79). They constitute a family of lectins which are synthesized in the cytosol, secreted unconventionally to the apical extracellular space, from where they are subsequently internalized into endosomal compartments and transported back to the lumen of the TGN (79). Most galectins form dimers or higher-order oligomers that contain multivalent carbohydrate-binding sites (80). The multivalent galectin-carbohydrate interactions have been hypothesized to induce clustering of glycoproteins and/or glycosphingolipids, and thus contribute to the apical sorting process upon vesicle formation from the TGN (78, 79). Some galectins, like galectin 3, specifically recognize apically-targeted glycoproteins, including some GPI-APs, to drive their apical delivery (78). Other galectins, however, have been shown to bind glycosphingolipids, such as sulfatides that carry long-chain-hydroxylated fatty acids (galectin 4) or the major apical glycosphingolipid in MDCK cells, called the Forssman glycolipid (galectin 9), and are stronger candidates to have a clustering function (77, 79). Interestingly, the GPI anchor of certain GPI-APs is modified with the addition of branched N-acetylgalactosamine (GalNAc) residues to the GPI-glycan (2), which could potentially be recognized by multivalent galectins. It is possible, then, that galectins can cross-link GPI-APs through their GPI-glycan moieties with the surrounding glycosphingolipids to promote the stabilization of GPI-AP clusters in domains enriched in glycosphingolipids, thereby facilitating their sorting into apically delivered vesicles. It would be interesting to explore this possibility.

Finally, it is still unclear whether specific transmembrane cargo-coat adaptors analogous to the p24 complex and cytosolic coat proteins are required for the formation of vesicles enriched in GPI-APs from the TGN. Several accessory factors, such as MAL/VIP17, annexins, flotillins, or stomatin, have been proposed to contribute to the apical sorting by promoting clustering of GPI-APs, as well as other apically-targeted proteins in lipid domains, although their mechanisms are still not clear (81–87). In addition, the Golgi-associated protein, FAPP2, through its PH domain, also seems to play a relevant role in apical sorting by mediating membrane deformation for generation of apical transport carriers (88).

CONCLUSIONS

Although there are differences between the sites and details of the process of sorting of GPI-APs in the secretory pathway in yeast and mammalian cells, there is an overall conservation of mechanism. GPI-glycan remodeling in the ER is conserved and is used to regulate the association of GPI-APs with p24 proteins, linking them to the formation of COPII vesicles and exit from the ER. In both cases, the lipid anchors are remodeled, although the exact modifications and locations are different, but this seems to play an important role in how the GPI-APs associate with the membrane and most likely helps to ensure GPI-AP concentration and sorting.



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